374-Pos Board B154
Effects of Cationic Residues and Base Sequence in Nucleic Acid Binding of Histone-Derived Antimicrobial Peptides
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Antimicrobial peptides (AMPs), which are found in numerous living organisms, are active against a wide range of bacteria and other pathogens. While many AMPs inhibit bacterial growth through membrane disruption, certain AMPs, including buforin II and DesHDAP1, are hypothesized to kill bacteria by binding to intracellular nucleic acids after translocating across the bacterial cell membrane. To understand this lesser known mechanism, the nucleic acid-peptide binding interactions of these systems were investigated using both experimental and computational methods. The nucleic acid-peptide binding for buforin II and DesHDAP1 were measured experimentally using a fluorescence intercalator displacement (FID) assay. In these experiments, having an increased composition of basic residues that are arginine versus lysine were shown to promote binding for variants of both buforin II and DesHDAP1. When binding was tested using nucleic acid sequences, neither buforin II nor DesHDAP1 significantly favored particular DNA or RNA sequences. To provide structural explanations for these results, molecular dynamics (MD) simulations and electrostatics calculations were used. Through these computational analyses, particular arginine residues within each peptide were found to interact more favorably with nucleic acids. Results from MD simulations also showed that nucleic acid-peptide binding was mostly due to interactions between the peptide and phosphate backbone of nucleic acids. Since these phosphate groups are identical for any DNA or RNA sequence, the prevalence of those interactions explained the lack of sequence specificity observed experimentally. These insights regarding nucleic acid binding of buforin II and DesHDAP1, paired with a deeper understanding of the peptides' structures and membrane interactions, are necessary to develop AMPs for novel pharmaceutical applications.

375-Pos Board B155
Effect of an Oxidative Guanine Lesion on Nucleosome Stability
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During cellular respiration, reactive oxygen species (ROS) escape from mitochondria to oxidize cellular components including DNA. Of all four DNA bases, guanine has the lowest redox potential, resulting in the oxidation of DNA to form 8-oxoguanine (8-oxoG). Because it has an even lower redox potential than guanine itself, 8-oxoG is likely to be even further oxidized. Of the potential products that can result from the oxidation of 8-oxoG, the formation of spiroiminodihydantoin (Sp) is of particular interest, as it appears to be even more mutagenic than 8-oxoG. Previous investigators demonstrated that the Sp lesion significantly destabilizes duplex DNA when compared to both its parent lesion 8-oxoG and guanine. As the structure of genomic DNA in chromatin is more complex, our goal is to examine the effect of the Sp lesion on nucleosome formation.
The Sp lesion is placed in specific locations along a 146-mer DNA oligonucleotide duplex, and this DNA is assembled onto chicken histones to form mononucleosomes by a double-dialysis method. Our data show that rapid nucleosome formation leads to kinetic trapping in non-equilibrium states. At equilibrium, the fraction of nucleosomes formed was the same for control and Sp-containing duplexes, regardless of lesion location or diastereomer. As the free energy of nucleosome formation was comparable despite the presence of the Sp lesion, we questioned whether the Sp lesion affects the rotational orientation of the proteins relative to the DNA. DNase I footprinting indicates distinctly different cleavage patterns both proximal to the lesion site and at locations as far as 30 base pairs away on the distal side of the core particle. We expect that additional footprinting experiments using diverse chemical reagents will allow us to more precisely define the positioning of the nucleosome on lesion-containing DNA.

376-Pos Board B156
Elucidation of DNA Packaging and Mispackaging in Sperm Nuclei by X-Ray Scattering
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DNA in vertebrate sperm nuclei is organized differently from any other cell. DNA is compacted in tightly packed (~ 400 mg/ml) hexagonal arrays. Since DNA repair is absent in sperm, dense packaging of DNA is considered necessary to protect against damage by reactive oxidizing species. DNA damage correlates with infertility and likely contributes to miscarriages and birth defects. Our goal is to characterize protamine defects that result in DNA mispackaging. Small (~30-50 amino acids), arginine rich (50-70%) peptides called protamines assemble DNA in sperm nuclei. Protamines will spontaneously condense DNA from dilute solution into close-packed, liquid crystalline arrays. Both sperm nuclei and reconstituted protamine-DNA arrays are sufficiently ordered that the interhelical spacing or packing density can be determined by x-ray scattering.
Insufficient protamine to neutralize all DNA has been linked to excess damage. Salmon sperm nuclei can be fractionated on sucrose density gradients. The interhelical scattering maxima of the heavy and light fractions overlap indicating that all nuclei have a tightly packed fraction of DNA. The lighter fraction, however, shows a scattering profile that is skewed toward larger spacings. Part of the DNA is not as well packed as the majority. Adding excess protamine to the light fraction recovers the scattering profile of the heavy fraction.
Protamines are initially serine phosphorylated when replacing histones on DNA. Incomplete dephosphorylation has also been linked to DNA damage. Herring protamines can be partially phosphorylated with protein kinase A. We find that even modest phosphorylation that reduces the protamine charge by only 5% will increase the water volume accessible to oxidizing species in reconstituted DNA-protamine assemblies by 50%.

377-Pos Board B157
Yeast HMGB Proteins Both Disrupt and Compact Nucleosomes
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High-mobility group (HMG) proteins are DNA binding proteins believed to play a significant role in reorganizing the conformation of chromatin, facilitating transcription, replication and DNA repair. Both HMO1 and Nhp6A are *Saccharomyces cerevisiae* HMGB family sequence-nonspecific DNA binding proteins containing HMG box motifs that generate strong bends in DNA. We are studying the hypothesis that binding of HMGB proteins disrupts chromatin, possibly opening binding sites for other factors. To study this architectural function, we have observed nucleosome conformation in complementary single molecule experiments involving atomic force microscopy (AFM) and optical tweezers (OT). In AFM experiments, nucleosome arrays are reconstituted and probed in liquid on mica substrates. Images reveal tightly compacted nucleosomes, which are characterized by small inter-core particle distances. Increasing concentrations of HMGB proteins initially increase average core particle distance, then decrease it as the DNA is compacted. OT experiments unfold arrays of nucleosome core particles, probing structural stability. OT data show that forces of 10 – 20 pN are required to fully disrupt nucleosome core particles, while experiments in the presence of HMGB proteins show unfolding at lower forces (at and below 10 pN). Taken together, these results indicate HMGB proteins alter chromatin structure, possibly by disrupting, but not fully displacing, nucleosomes.

378-Pos Board B158
The Effect of HU Protein on Lac-Repressor-Mediated DNA Looping
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The regulation of transcription includes the formation of DNA loops mediated by proteins that bind to DNA. DNA stiffness and supercoiling influence DNA loop-formation and can be modified by abundant nucleoid associated-proteins in bacteria that bind to DNA. Studies indicate substantial redundancy between different nucleoid proteins *in vivo*, but deletion of the heat unstable protein (HU) makes the formation of short loops between strong recognition sequences dependent on the sequence of the loop segment. The HU protein has two subunits (α and β), induces negative supercoiling of DNA, and changes the flexibility of DNA upon binding non-specifically. For loops longer than a persistence length, for which DNA stiffness is not limiting, the effect of HU on loop formation is not well understood. In tethered particle motion (TPM) experiments, a titration of the formation of a 900 bp loop between strong recognition sequences, O_{ID} and O_1, as a function of HU concentration exhibits mild changes across a broad range of concentration (0-900 nM). This was surprising since the overall tether length simultaneously decreased which should have facilitated looping. It suggests that HU has no specific effect on the *lac* repressor-induced DNA loop, the effect of HU is more prominent on shorter loops, or the strong O_{ID} operator, which supports up to 90% looping at a variety